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Injury, reflex impairment, and survival of beam-trawled flatfish

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Under the "high survival" exemption of the European landing obligation or discard ban, monitoring vitality and survival of European flatfish becomes relevant to a discard-intensive beam trawl fishery. The reflex action mortality predictor (RAMP) method may be useful in this context. It involves scoring for the presence or absence of natural animal reflexes to generate an impairment score which is then correlated with post-release or discard mortality. In our first experiment, we determined suitable candidate reflexes for acclimated, laboratory-held European plaice (*Pleuronectes platessa*) and common sole (*Solea solea*). In a second experiment, we quantified reflex impairment of commercially trawled-and-handled plaice and sole in response to commercial fishing stressors. In a third experiment, we tested whether a combined reflex impairment and injury (vitality) score of plaice was correlated with delayed post-release mortality to establish RAMP. Five-hundred fourteen trawled-and-discarded plaice and 176 sole were assessed for experimentally confirmed reflexes such as righting, evasion, stabilise, and tail grab, among others. Of these fish, 316 plaice were monitored for at least 14 d in captivity, alongside 60 control plaice. All control fish survived, together with an average of 50% (± 29 SD) plaice after being trawled from conventional, 60 min trawls and sorted on-board a coastal beam trawler. Stressors such as trawl duration, wave height, air, and seawater temperature were not as relevant as a vitality score and total length in predicting post-release survival probability. In the second experiment where survival was not assessed, reflex impairment of plaice became more frequent with prolonged air exposure. For sole, a researcher handling-and-reflex scoring bias rather than a fishing stressor may have confounded results. Scoring a larger number of individuals for injuries and reflexes from a representative selection of trawls and trips may allow for a fleet-scale discard survival estimate to facilitate implementation of the discard ban.

Keywords: animal behaviour, common sole (*Solea solea*), discard mortality, European plaice (*Pleuronectes platessa*), reflex action mortality predictor, vitality.

Introduction

Despite a ban dictated by the European Common Fisheries Policy (European Union, 2013), discarding fish and crustaceans at sea may still be allowed if they have a high chance to survive. Reliable methods are needed to quantify this (unaccounted) survival or mortality probability to facilitate implementation of the landing obligation. One promising method may be the reflex action mortality predictor (RAMP; Davis, 2005, 2010; Davis and Ottmar, 2006); an approach that has revealed strong correlations between an organism's innate capability to respond to reflex stimuli (an index of its vitality) and its probability of surviving fishing capture and release (Barkley and Cadrin, 2012; Raby *et al.*, 2012).

Reflex assessments cover the neuro-muscular response system of an organism's body, and are independent of gender, size, or context-specific behaviours such as competition. They can also integrate relevant injury types by scoring symptoms of barotrauma, skin damage, bleeding, or bruising (Davis, 2010; Cooke *et al.*, 2013; Nguyen *et al.*, 2014; Benoît *et al.*, 2015). Mainly for these reasons, and also for being less invasive, costly, and laborious than other methods such as bio-acoustic telemetry and blood sampling, RAMPs have been applied in the field to fish (Davis, 2010; Barkley and Cadrin, 2012), crustaceans (Stoner, 2012; Yochum *et al.*, 2015), and reptiles (Ledain *et al.*, 2013; Stacy *et al.*, 2013). Another advantage is their practicality in being taught to researchers (Cooke *et al.*, 2013). Responses to reflex stimuli and occurrence of injury types are scored as either present or absent and coded as binary variables (i.e. "0" or "1"; Kestin *et al.*, 2002, Davis, 2010). This removes more subjective interpretations of reflex strength or severity of injury.

Scoring fish responsiveness to reflex stimuli as an index of vitality had been practiced in veterinary medicine to assess brain function (Kestin *et al.*, 2002) and in biological sciences in search for reliable proxies of stress and post-release mortality (Davis, 2007, 2010; Barkley and Cadrin, 2012). Impairment may be expressed as the proportion of impaired reflexes over all those assessed (Davis, 2010; termed impairment score); including injuries in this score generates a vitality score. Failure of reflex actions such as the ability to roll eyes or lift the operculum, may be an indication that the brainstem has become insensible (Kestin *et al.*, 2002). Whereas, disorientation or inability of the body to move may be due to muscular fatigue and metabolic deficits as a consequence of a fish being chased, crowded and exhausted during capture (Wardle, 1983), or being stressed during on-board handling from hypoxia, thermal, or haline shocks (Broadhurst *et al.*, 2006).

In wild capture fisheries, multiple stressors may influence fish mortality probability and the proportion of reflex impairment and injury may change over a linear gradient of stressors (Davis, 2007). In such a case, reflex and injury scoring may be predictive of post-release mortality once important stressors are included in RAMP assessment (Davis, 2010; Barkley and Cadrin, 2012). If reflex impairment and injury presence of commercially caught-and-discarded European flatfish was indicative of their post-release fate than a large number of individuals from a wide range of fishing conditions may be scored for their vitality during existing at-sea data collection programmes (ICES, 2015). These data may be used as a proxy for survival when combined with observations from captive or tagged animals and may facilitate implementation (or otherwise) of the discard ban.

Many beam trawl fishers are confident that European flatfish such as European plaice (*Pleuronectes platessa*) and common sole (*Solea solea*) are sufficiently robust to survive beam-trawl capture and discarding. A "high survival" exemption from the otherwise obligatory retention of all sizes may facilitate industry viability. Indeed, there is evidence that some plaice and sole do survive beam trawling, although some of the earlier estimates have been low (<15%, Van Beek *et al.*, 1990). Both species lack a swimbladder and may tolerate some stress and physical damage from capture and discarding, even by heavy beam trawls (Revill *et al.*, 2013; Depestele *et al.*, 2014a). As found for other flatfish species for which reflex tests were successfully carried out (e.g. Northern rock sole [*Lepidopsetta polyxystra*], Davis and Ottmar, 2006; yellow-tail flounder [*Limanda ferruginea*], Barkley and Cadrin, 2012), we hypothesized that plaice and sole exhibit reflexes that are sensitive to some common stressors during the capture-and-handling process of commercial beam trawling, and that the probability of post-release survival is associated with the degree of reflex impairment and presence of certain injury types such as scale loss, bruising, and point bleeding.

Based on the logic above, our objectives here were to (i) identify suitable candidate reflexes for plaice and sole in the laboratory (Experiment 1), (ii) test *in situ* their sensitivity towards gradients of stressors (differentiated by two beam trawl gear types and three air exposure periods during commercial trawling and handling; Experiment 2), and (iii) describe the relationship between vitality scores and fishing stressors (e.g. trawl duration) with the observed post-release survival of plaice (Experiment 3).

Material and methods

Ethics statement

This research was done in a research laboratory in Belgium (Experiment 1) and on-board commercial beam trawlers in the Southern North Sea (Experiments 2 and 3) in accordance with the Institute for Agricultural and Fisheries Research (ILVO) scientific research permits. Relevant maritime authorities issued further permits for researchers to board commercial vessels and to keep undersized fish on-board. This study did not involve endangered or protected species. Animal ethics approval was granted by ILVO's Animal Care and Ethics Committee (EC2014/226).

Experiment 1

In Experiment 1, plausible candidate reflexes (Depestele *et al.*, 2014b; and P. Randall, pers. comm.; Supplementary Video S1) were tested on 18 healthy individuals of each plaice and sole (both males and females) for consistent reflex responses at a research laboratory in Ostend, Belgium (see Supplementary Video S1). Fish were sourced from <20 min research beam trawls on-board the RV Simon Stevin and during shore-based sampling (following Beyst *et al.*, 2001) in the North Sea during April 2014. The full size range found in the Belgian beam-trawl fishery (between 5 and 40 cm total length [TL]) were represented. All fish were acclimated in lab-based 124-l monitoring tanks (75 cm L × 55 cm W × 30 cm H) with a seawater recirculation system at ambient temperature (described by Soetaert *et al.*, 2015a) for at least 3 weeks. All 18 fish from each species were scored by two researchers on consecutive days; under the assumption of complete recovery within 24 h.

Candidate reflexes were tested in this order: (i) body flex, (ii) righting, (iii) vestibular-ocular response (i.e. eye roll), (iv) head, (v) evasion, (vi) stabilize, (vii) resistance, (viii) operculum, (ix) mouth, (x) startle touch, and (xi) tail grab (following Depestele *et al.*, 2014b; Supplementary Video S1). First, fish were held ventral side up on the palm of the experimenter's hands above a 30-l water-filled container, and scored for an upwards flexing of the head and tail (body flex). Then, they were released into the container to see whether they swam to the bottom by righting themselves. Next, the movement of the eye ball was closely monitored while the fish was rotated around its lengthwise axis out of water. A healthy fish kept the eyes focused in a plane, whereas eyes of an impaired fish followed the movement of the body (eye roll). The head reflex was scored as impaired, when the fish dangled motionless while being held by its head between thumb and index finger with its belly side up. In contrast, an unimpaired fish actively curled its body around the experimenter's fingers. For the evasion reflex, fish were held at the water surface between both hands with the dorsal side facing up to see whether it was able to actively swim away upon release. Once the fish reached the bottom of the container, the stabilize reflex was scored as unimpaired when its dorsal fins and/or its body moved rhythmically as if it was digging itself into sand. Placing a hand flat across the fish on the bottom any resistance movement to the constraint was scored as unimpaired. Its head complex was examined using a blunt object (such as a pencil) to gently lift the operculum on a restrained fish. Resistance to lift it or a clamping movement to close it were signs of an unimpaired fish. Similarly, the mouth was opened and any resistance or closing movement was scored as unimpaired. Startle touch was assessed by striking the dorsal fins with the pencil to elicit a reaction. For the tail grab, fish were gently grabbed by their tails between thumb and index finger. An unimpaired fish struggled free and actively swam away. All fish were monitored daily for at least 10 d thereafter.

Experiment 2

Two field experiments were done involving commercial beam trawlers in the Southern North Sea. The aim of Experiment 2 was to evaluate the sensitivity of those candidate reflexes selected from Experiment 1 when exposed to stressor gradients. We tested the influence of air exposure during sorting and impact from trawl capture either with tickler chain or pulse trawl gears. This experiment was done in June 2014 on-board two commercial Dutch beam trawlers targeting sole with double-rigged 12-m sumwing beam trawls with a 80 mm stretched mesh opening. One vessel used 20 conventional metal tickler chains (4.5–26 m in length, and 16–24 mm chain link diameter), whereas the other vessel used 12 HFK-engineered electric pulse electrodes (60 Hz pulsed bipolar current at 45–50 V with a 0.36 ms pulse duration; Soetaert *et al.*, 2015b). The following technical, environmental, and biological data were recorded for each trawl: start and end positions, trawl duration; wind force; average water depth; and catch weight. Catch weights were estimated in kg and where necessary "number of boxes" converted to weights by a factor of 35 kg. Additional measurements such as maximum wave heights, air, and surface seawater temperatures were received from nearby weather stations "Trapegeer", "Westhinder", and "Wandelaar", respectively (Flemish Government, 2015). For each sampled trawl, batches of between 8 and 12 plaice or sole across their size range (including specimens > MLS) were collected from the sorting conveyor at the beginning, mid-, and end-points of the sorting process, placed into a 10-l ambient seawater-filled PVC bucket and within ~5 min tested for their reflexes (by two different observers); except for the resistance and startle touch reflexes. Species-specific reflexes included body flex that was exclusively tested on plaice and head on sole. Air exposure for each fish was expressed as the minutes between the time the catch was decked and a fish being placed inside the water-filled bucket plus one-third of the handling time during reflex testing (because one-third of the reflex tests were done in air out of the water-filled container).

Experiment 3

The aim of Experiment 3 was to establish whether combined reflex impairment and injury (i.e. vitality) of plaice or any other relevant fishing stressor was associated with survival. Five <16-h trips were done with a commercial Belgian beam trawler (Eurocutter fleet segment) targeting sole in shallow (average depths of 12 m ± 5 m, SD) coastal waters of the Southern North Sea between November 2014 and September 2015 following the same data collection protocols as described for Experiment 2 (Table 2). Controls were sourced from the RV Simon Stevin in August/September 2014 and May 2015 as in Experiment 1 above. The beam trawler was conventionally rigged with two 4-m beam trawls with chain mats (each weighing ~1300 kg) and 80 mm diamond mesh codends. Plaice were assessed for their delayed mortality inside stacks of independently arranged, water-filled, and on-board 30-l monitoring containers (60 cm l × 40 cm W × 12 cm H; Figure 1) for ~15 h and then transferred within <2 h to laboratory-based, 124-l monitoring containers for 14 d of at least daily monitoring. On-board each monitoring container was stocked with five fish from the same picking location and trawl at a density <5 kg m^{-2}. To study the effect of trawl duration, during each trip, one or two short (15–20 min) trawls were done in between conventional trawls (typically ~60 min, but ranging between 15 and 120 min depending on conditions, D. Torcq, pers. comm.). Plaice were randomly picked in batches of five fish from the sorting conveyor as described in Experiment 2, except for an extra batch collected straight from the hopper (a large container holding the discharged catch after the codend was emptied). Each plaice was scored by the same observer on all trips for the body flex, righting, evasion, stabilize, and tail grab reflexes, and a presence/absence of injury (e.g. extent of bruising around head or body). These injuries were associated with mortality in earlier work (Depestele *et al.*, 2014a;

Figure 1. On-board monitoring rack (152 cm l × 59 cm W × 160 cm H) with 16 independently mounted, flow-through 30-l monitoring containers (60 cm l × 40 cm W × 12 cm H).

J. Depestele, pers. comm.). All reflex-tested fish were length measured to the nearest cm of TL and T-bar (29 × 8 mm) anchor tagged with Bano'k© guns in the dorsal musculature following McKenzie *et al.* (2012). Fish were offered defrosted brown shrimp (*Crangon crangon*) as food at <5% of their biomass after 7 d of monitoring. Any food remains and/or dead fish were removed and the time to mortality noted. Fish were monitored three times per day within the first and daily within the second week of monitoring.

Analyses

Following Davis (2010), a response to a reflex stimulus was scored as present (unimpaired, 0) when clearly visible, or absent (impaired, 1) when not visible, weak or in doubt within 5 s of observation. The corresponding impairment score for each fish was calculated as the mean score of impaired reflexes (and present injuries—in Experiment 3). Reflex and injury assessments took ~30 s per fish.

In Experiment 2, the TLs of reflex-tested plaice and sole were compared between gear types with a linear-mixed model (LMM) with gear as fixed effect and trawl as random effect. In Experiment 3, an LMM was fitted to the TL of sampled plaice including trawl duration (continuous) and picking location (categorical) as either directly from the hopper or sorting conveyor as fixed effects and trawl as random effect.

For the data of Experiment 2, gear type and air exposure (i.e. sampling at the beginning, mid-, and endpoints of the sorting process) were used as categorical explanatory variables (fixed effects) in a multivariate permutational ANOVA (PERMANOVA) analysis to test for their influence on all measured binary reflex responses using the Plymouth Routines In Multivariate Ecological Research (PRIMER) programme, version 6.1.12 with PERMANOVA add-on software (Anderson *et al.*, 2008). To construct a similarity matrix, the simple matching similarity index was used, because it includes joint absences of reflex impairment. A univariate PERMANOVA was done with reflex impairment score as the response variable with the same two-factorial design building a similarity matrix based on Euclidean distance. Statistical significance of results were considered at $p < 0.05$.

In Experiment 3, survival as a function of time was visualized using a non-parametric Kaplan–Meier function. The effect of vitality score and length was analysed using a semi-parametric Cox-proportional hazards model with vitality score and length as fixed effects using the coxph-function in the survival package (Therneau, 2015) from the freely available R software (version R 3.2.2, R Core Development Team, 2015).

To determine whether vitality score among other relevant technical, environmental, and biological factors were associated with survival probability, a cross sectional approach to data analysis was chosen. A generalized linear-mixed model (GLMM) with a random intercept for trawl and monitoring container was fitted to the survival status of fish after 14 d of monitoring in Experiment 3. All data were explored following the protocol by Zuur *et al.* (2010) checking for outliers and relationships between variables. Wave height was log-transformed to account for the rather extreme peaks of 1.8 m waves during the second trip. To find the optimal set of covariates an information theory approach was followed (Burnham and Anderson, 2002). After checking for collinearity, the following variables were considered in *a priori* models for a fish surviving for up to 14 d after being discarded: vitality score, TL, trawl duration, air exposure, wave height, and the temperature difference between seawater and air. All combinations of these covariates, including biologically plausible two-way interactions, except for impairment score and TL, were tested in 78 candidate models. The most parsimonious model was validated by checking how reliably it would predict mortality events from one-third of the randomly split dataset. To obtain the fits, the lmer4 package (Bates *et al.*, 2015) was used in R. A significance level of $p = 0.01$ was used.

Results

Experiment 1

Consistent reflex responses were identified among healthy plaice (Figure 2a) and sole (Figure 2b) from the original candidate reflexes, including the body flex, righting, head, evasion, stabilize, mouth, operculum, and tail grab reflexes. None of the fish died. Some species-specific differences were observed: in contrast to sole,

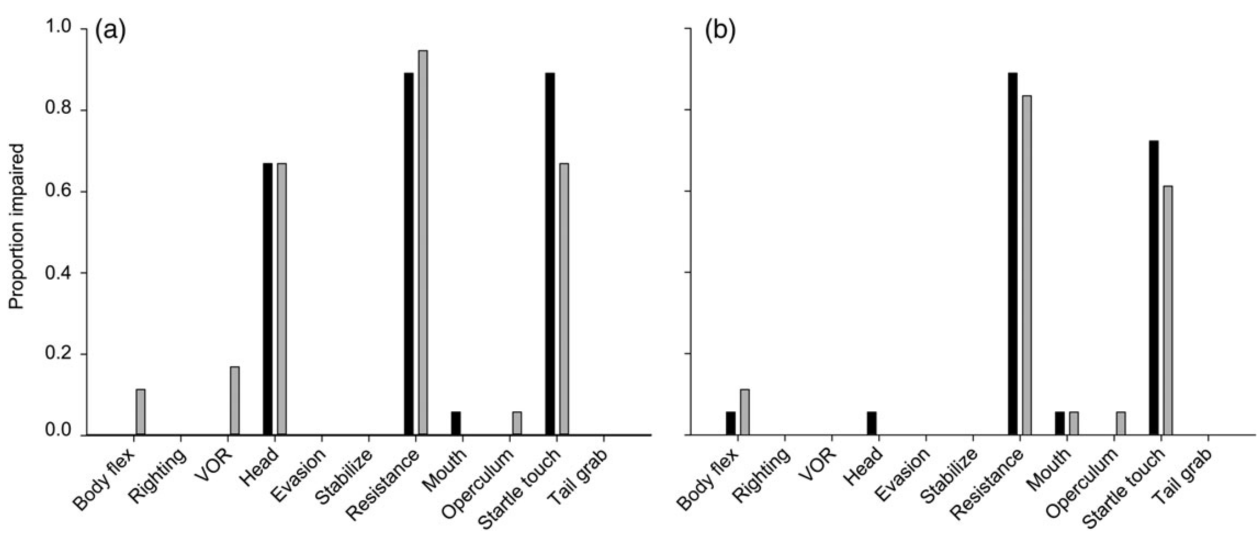

Figure 2. Consistency of one observer (black bars) and a fellow observer (grey) in recognizing impaired candidate reflexes of healthy, lab-acclimated (a) plaice (*P. platessa*; $n = 18$ fish) and (b) sole (*S. solea*; $n = 18$ fish). Reflexes such as resistance, startle touch (both species), and head (plaice only) with proportions of impairment >0.5 were considered unsuitable candidate reflexes.

plaice were unresponsive to the head reflex. The eye roll seemed to be the most difficult one to consistently recognize between the two initial observers. Due to some deviation in agreement between researchers and foreseen difficulties to test the eye roll reflex under unstable conditions on-board moving vessels, it was excluded from the reflex data of Experiment 2.

Experiment 2

One hundred ninety-eight plaice and 176 sole were scored for reflex impairment from 16 trawls. According to the LMM, TL (mean ± standard error [SE]) of pulse-caught plaice ($n = 110$) and sole ($n = 85$; 25.4 cm ± 0.5 cm and 24.8 cm ± 0.5 cm, respectively) were significantly smaller compared with reflex-tested plaice ($n = 88$) and sole ($n = 91$) caught by the tickler-chain gear (28.0 cm ± 0.5 cm and 27.7 cm ± 0.5 cm TL, respectively; $p < 0.001$). Fishing conditions on both vessels were similar, apart from catch volumes (Table 1). On-board the pulse trawler, catches were on average at least half that of tickler-chain trawls, resulting in quicker sorting times and hence, shorter air exposures (Table 1; Figure 3a and b). Each fish spent 3.3 ± 2.5 (mean ± standard deviation, SD) min inside the bucket before being reflex tested, but compared with the tickler-chain-equipped trawler on-board the pulse trawler sole remained on average 1 min longer inside the 10-l bucket before being tested for reflexes (Table 1), because incidentally more sole were sampled per batch on-board the pulse compared with the tickler-chain-equipped trawler, delaying processing times of the fish.

For plaice, reflex impairment significantly increased with increasing air exposure on both vessels, but was independent of gear type. Median reflex impairment scores increased with a collection from the sorting conveyor towards the mid- or endpoint of the sorting process (d.f. = 2, $p < 0.01$; Figure 3a). Further, individual reflexes were more frequently impaired among fish being collected later in the sorting process (d.f. = 2, $p < 0.01$; Figure 4a). For sole, reflex impairment scores (of both individual and all reflexes combined) were less affected by air exposure during sorting. Instead impairment was significantly different between tickler-chain and pulse trawls, with fewer fully impaired fish sampled on-board the pulse vessel (d.f. = 1, $p < 0.01$ and $p < 0.05$ for individual and combined reflexes, respectively; Figures 3 and 4b).

Table 1. Summary of mean ± SD key technical, environmental, and biological variables collected on-board the tickler-chain equipped or pulse trawler (n observations) in Experiment 2.

Variable	Tickler-chain trawls	Pulse trawls
Month	June	June
Total no. of deployments	30	21
Deployments sampled		
For plaice	7	4
For sole	8	4
No. of plaice sampled	88	110
No. of sole sampled	91	85
Technical		
Trawl		
Depth (m)	23 ± 1 (9)	25 ± 2 (7)
Duration (min)	107 ± 5 (9)	111 ± 8 (7)
Environmental		
Wind force	2 ± 1 (9)	2 ± 1 (7)
Air exposure (min)		
Begin of sorting	13 ± 4 (60)	4 ± 0 (65)
Midpoint of sorting	21 ± 5 (59)	14 ± 2 (67)
Endpoint of sorting	30 ± 10 (60)	19 ± 1 (63)
Handling time for plaice (min)	3 ± 2 (92)	3 ± 2 (110)
Handling time for sole (min)	3 ± 2 (91)	4 ± 4 (85)
Biological		
Total catch (kg)	1599 ± 439 (9)	601 ± 203 (7)
Trawled plaice total length (cm)	28 ± 4 (92)	25 ± 5 (110)
Trawled sole total length (cm)	28 ± 4 (91)	25 ± 5 (85)

Experiment 3

Three hundred and seven plaice (22 ± 4 cm TL, mean ± SD) scored for reflex impairment and the presence of injuries and monitored for at least 14 d after being "discarded" into monitoring containers during five trips (Figure 1) were analysed. Fish that lost their tag ($n = 3$) and which were not measured for reflexes (5) nor length (1) were excluded. During three trips, 60 controls (with 40 length-measured fish, 22.9 ± 0.6 cm TL, mean ± SE) were monitored alongside treatment fish (Table 2). Plaice sampled from the sorting conveyor ($n = 205$) were significantly larger (21.9 ± 0.2 cm, mean ± SE) than fish ($n = 102$) taken straight from the hopper (20.8 ± 0.4 cm, mean ± SE) (LMM, $p < 0.05$). Seawater temperature and wave height varied between trips and to account for potential temperature shocks for fish being hauled from the bottom to the surface and being exposed to air during sorting, the difference between sea surface and air temperature was considered as an additional explanatory variable (Table 2). Environmental conditions in the laboratory-based monitoring tanks were comparable to those during trawling with 1 ± 3°C (mean ± SD) differences in seawater temperatures between the on-board and laboratory-based containers. Due to adverse weather during the second trip, air exposure was approximated for 14 plaice, assuming that sampling at the beginning, mid-, and endpoints of sorting occurred 5, 12, and 19 min after the net came on deck, which was the average delay in collecting other fish during sorting on this trip. An average of 50% (± 29 SD) of 217 fish sampled from conventional trawls survived for 14 d, compared with 75% (± 13) of 99 plaice from ≤20-min short trawls (Figure 5). None of the 60 control fish died (Figure 5). Mortalities began to cease within 5–14 d after capture (Figure 5). Except for the third trip, there were fewer mortalities (up to 10 times) among fish sampled from short compared with conventional trawls (Figure 5). Mortality increased with increasing vitality score, mortality decreased with increasing length. The corresponding hazard ratio of the vitality score and length from the Cox-proportional hazard model was 85 (95% confidence interval: [34.5; 210.3]) and 0.88 (95% confidence interval: [0.83; 0.92]).

Based on the GLMM, the most important variables to estimate the survival probability were a vitality score and TL (Tables 3 and 4). Fish <22 cm and those with vitality scores >0.39 were more likely to die (mean ± SE of dead fish: 20.7 ± 0.3 cm TL and 0.56 ± 0.02 vitality score vs. alive fish: 22.1 ± 0.3 cm TL and 0.39 ± 0.02 vitality score; GLMM, $p < 0.001$; Table 4; Figure 6). Including any of the other above variables resulted in models with similar explanatory power (Akaike weights $\omega_i > 0.05$; Table 3). In 52% of the cases, model M1 including the main effects for vitality score, TL, trawl duration, wave height, temperature difference, and an interaction between trawl duration and wave height was the optimal model (Table 3). This model was able to correctly predict observed mortality in 83% of the cases when applied to the validation dataset. Vitality scores, TL, trawl duration, and the interaction between trawl duration and wave height was

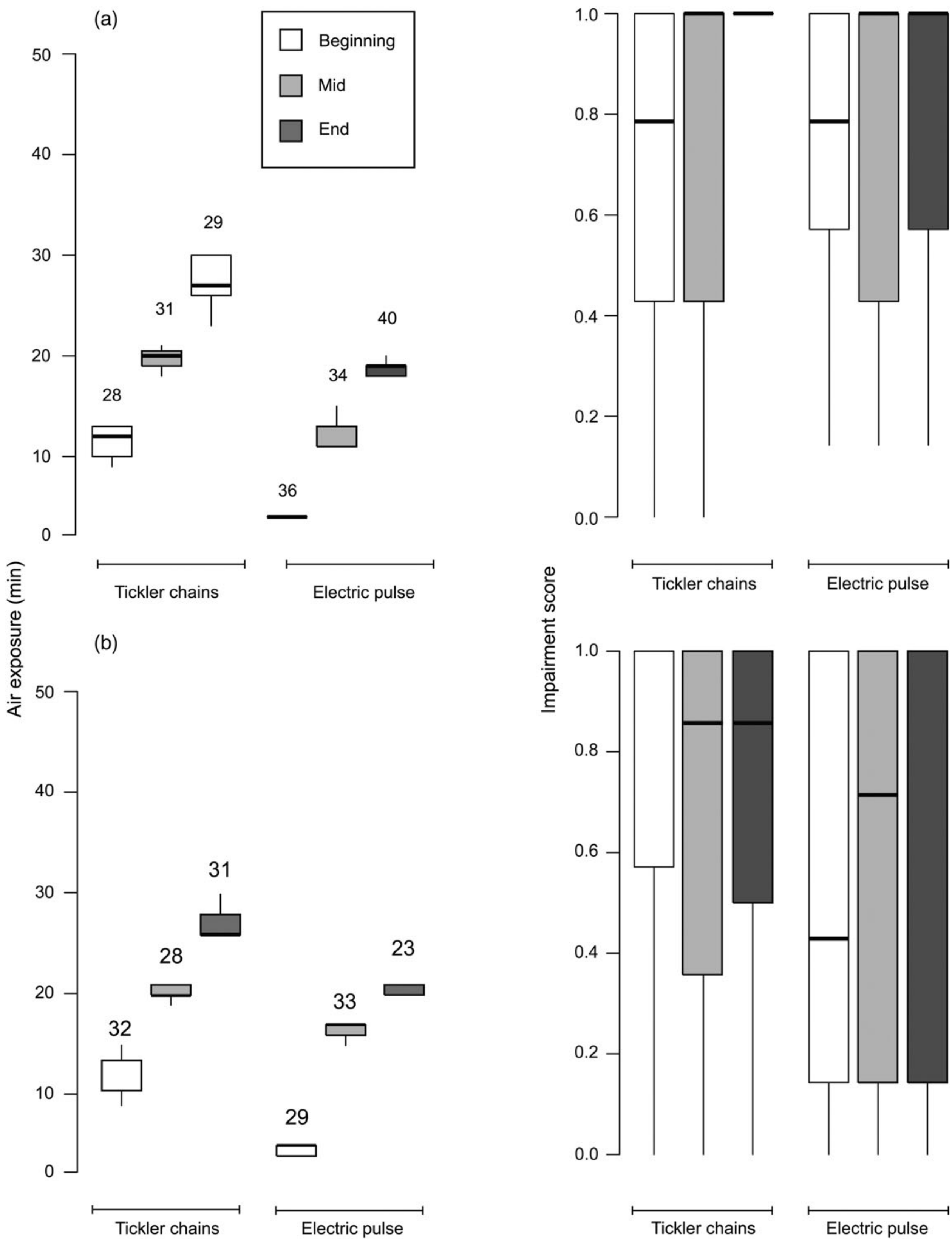

Figure 3. Air exposure (in min) and reflex impairment scores of (a) plaice (*P. platessa*; $n = 198$ fish) and (b) sole (*S. solea*; $n = 176$ fish) between the 10th and 90th percentile sampled at the beginning (white), mid- (light grey), or end-point (dark grey) of the sorting process on-board the tickler-chain equipped or electric pulse beam trawler in Experiment 2. The impairment score of fully impaired individuals equals 1.

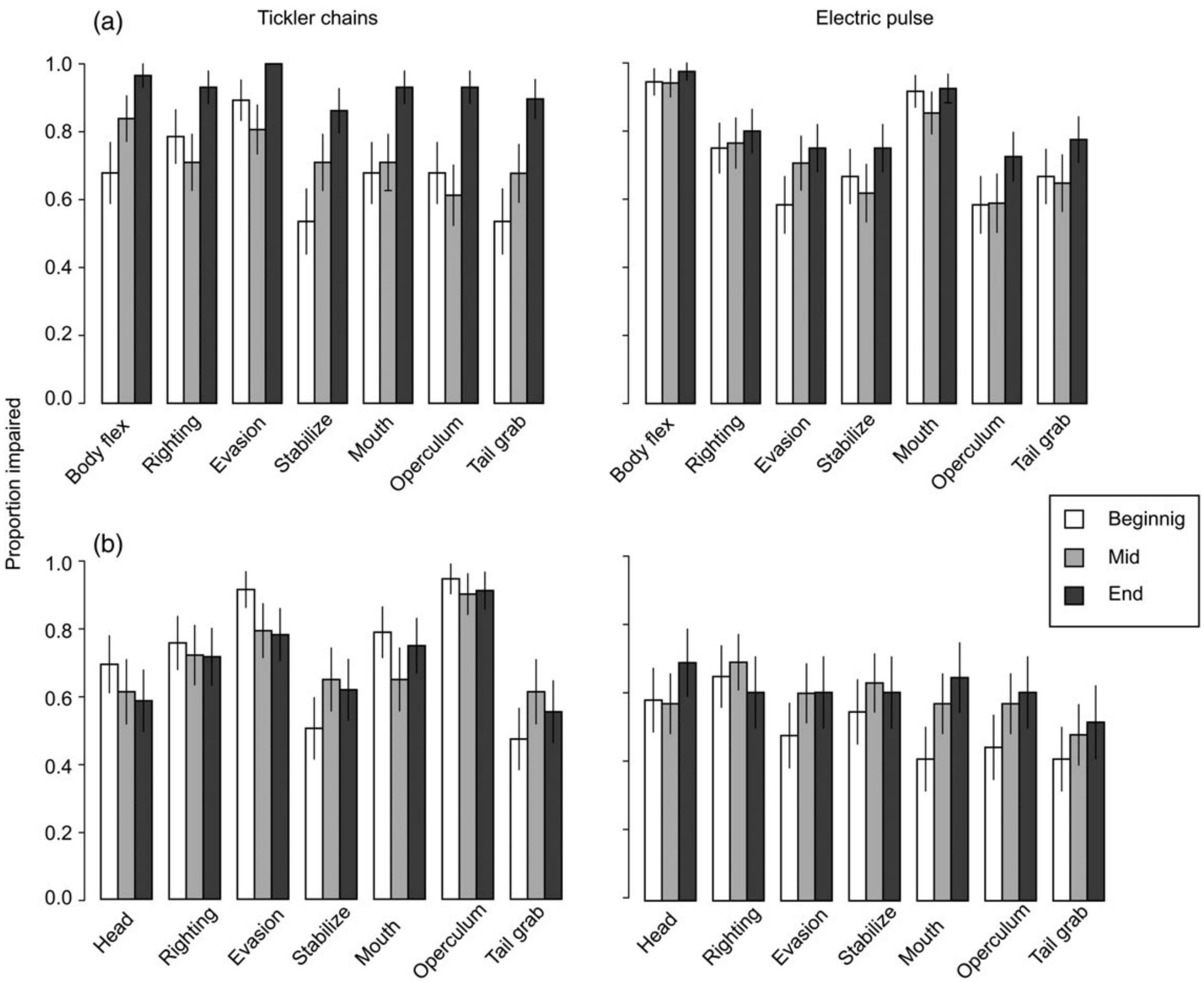

Figure 4. Proportion (mean ± SE) of impaired reflex responses of (a) plaice (*P. platessa*; $n = 198$ fish) and (b) sole (*S. solea*; $n = 176$ fish) sampled at the beginning (white), mid- (light grey), or end-point (dark grey) of the sorting process on-board the tickler-chain equipped or electric pulse beam trawler in Experiment 2.

significantly different from 0 at the 1% level in all models; temperature difference in models M1 and M2 (GLMM; $p < 0.01$; Table 4).

Discussion

The identified candidate reflexes of plaice and sole were sensitive to commercial fishing stressors, and a vitality score for plaice was significantly correlated with their post-release mortality. This finding supports the utility of RAMP as a predictor of flatfish post-release mortality (Barkley and Cadrin, 2012) and descriptor of fish vitality after commercial fishing capture (Raby *et al.*, 2012; Nguyen *et al.*, 2014). Survival probabilities of plaice were within upper ranges of earlier estimates for this species from studies with comparable monitoring periods (31–52% after 7 d, Kelle 1976; 20–57% after 3 d, Revill *et al.*, 2013; 48–69% after ≤3 d, Depestele *et al.*, 2014a). The observed results are temporally and spatially explicit and restricted to a coastal vessel which deployed its gear for ~60 min with <1 t catch weights. These conditions may be considered mild compared with ~120 min trawls (Poos and Rijnsdorp, 2007) and >5 t catch weights (Ulleweit *et al.*, 2010) that are typical for the majority of the beam-trawl fleet in the North Sea. Nevertheless, our results are indicative of the effects of relevant stressors on the vitality and short-term fate of discards (excluding effects of predation, Raby *et al.*, 2014).

Survival probability was highly variable between trips and also between short, <20-min and conventional, ~60-min trawls owing to synergistic or cumulative effects of prevailing technical, environmental, and biological conditions. Except for the interaction between trawl duration and wave height, most of the individual technical and environmental stressors (e.g. air exposure) were not clearly correlated with survival probability (comparable with results by Van Beek *et al.*, 1990; Revill *et al.*, 2013). Trawling in adverse weather exacerbated effects, and contributed to observed variability in survival probabilities between trips (e.g. wave heights exceeded 1 m during the second and fifth trip). However, recorded conditions during the third trip did not explain fewer mortalities from conventional compared with the short (i.e. 15 min) trawl. Thus, synergistically, the above and possibly some unrecorded factors affected fish vitality: less vital plaice were more likely to die. Similar relationships were established for trawl-caught yellowtail flounder (Barkley and Cadrin, 2012) and Pacific halibut (*Hippoglossus stenolepis*; Davis, 2007) with vitality scores explaining the variability among observed survival rates. Based on the

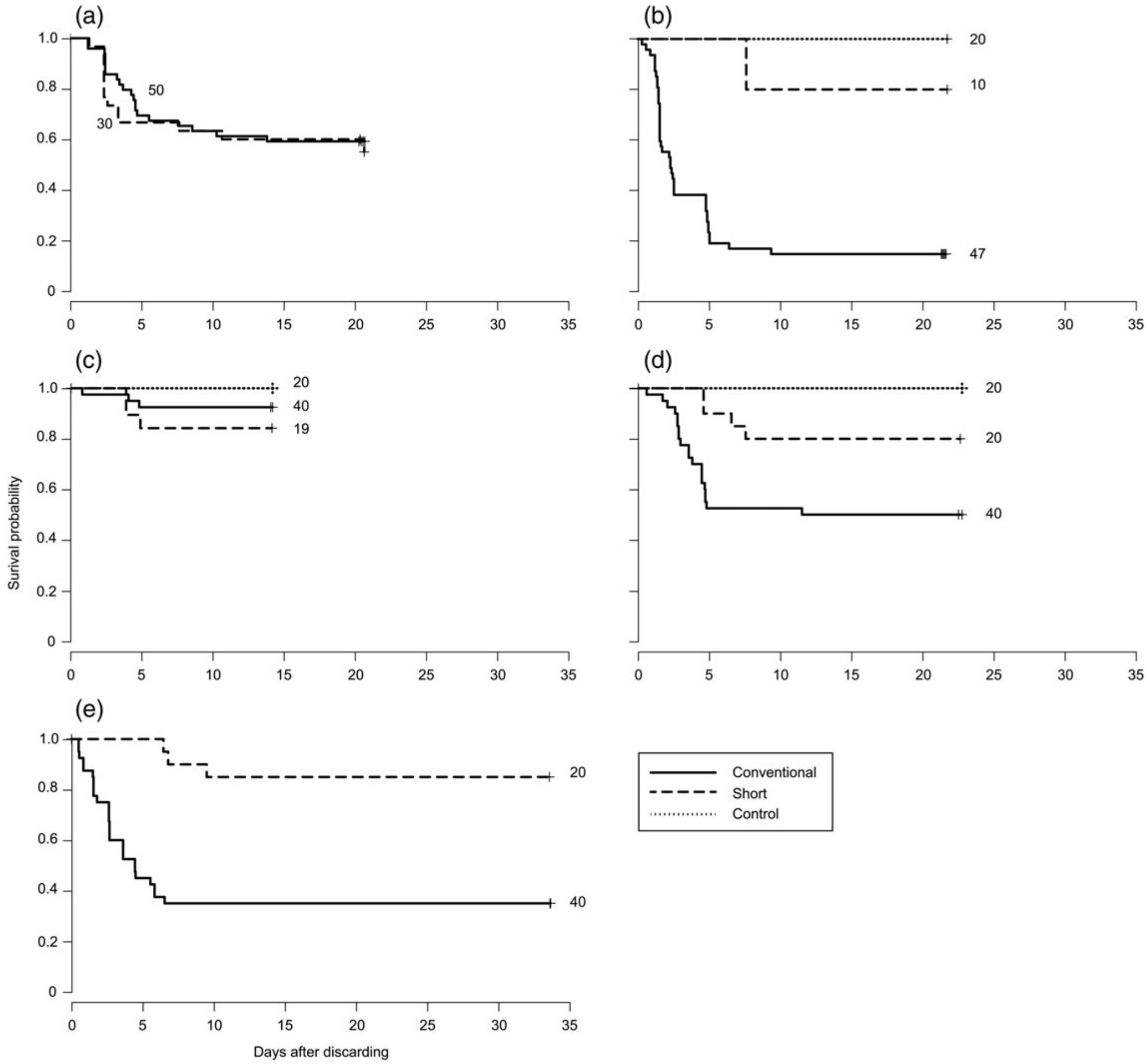

Figure 5. Non-parametric Kaplan–Meier survival probability estimates over days of monitoring of discarded plaice (*P. platessa*) collected during five trips (a-e) from conventional, 60-min (solid line), or short, <20 min (dashed line) trawls by a coastal beam trawler in Experiment 3. None of the control fish died (dotted line, b–d). Numbers indicate sample sizes.

relationship between vitality and plaice survival, results from the current study may be used to extrapolate survival rates from sample to trawl, trip, vessel, or even fleet level, as long as representative numbers of animals can be assessed for their impairment and injury during comparable fishing and environmental conditions (Benoît *et al.*, 2012; Yochum *et al.*, 2015).

While vitality was negatively correlated with post-release survival, TL had a positive relationship with the latter. In the current study, for instance, larger fish were more resilient towards capture stress than smaller conspecifics. Individuals with an average TL of 22 cm were less likely to die, supporting previous observations for plaice (Kelle, 1976; Revill *et al.*, 2013) and sole (Depestele *et al.*, 2014a). Larger fish may handle the herding and capture process inside the trawl better physically, become less exhausted than small fish and reserve energy (Wardle, 1978). Likewise, these larger plaice may also be able to handle confinement stress at a physiological level better than smaller fish. In turn, smaller fish may be more prone to suffer from their injuries given their lower mass in relation to body size (Broadhurst *et al.*, 2006; Revill *et al.*, 2013).

Notwithstanding the above, the relationship between vitality, fish size, and survival probability may be species specific (Davis, 2010). Compared with plaice, the aforementioned relationship among sole may be different owing to species-specific traits, adaptations, and physiological mechanisms to cope with any injury and stress experienced during commercial beam trawling (Shephard, 1994; Benoît *et al.*, 2013; Glover *et al.*, 2013). For example, compared with plaice, sole seemed more resilient towards hypoxia during on-deck sorting in Experiment 2, showing no increase in impairment despite prolonged air exposure times. In contrast, anecdotal observations by Kelle (1976) suggested that sole rather than plaice were less tolerant towards air exposure on deck. The reduction of

Table 2. Summary of mean ± SD key technical, environmental, and biological variables collected during each monitored trip of a commercial coastal beam trawler (*n* observations) in Experiment 3.

Variable	Trip 1	Trip 2	Trip 3	Trip 4	Trip 5
Month	November	December	February	June	September
Total no. of deployments	16	15	15	15	15
Short deployments sampled	2	1	1	1	1
No. plaice sampled	30	10	19	20	20
Conventional deployments sampled	4	5	2	2	2
No. plaice sampled	50	47	40	40	40
Technical					
Trawl					
Depth (m)	8.8 ± 2.7 (16)	16.1 ± 4.1 (15)	9.6 ± 3.6 (15)	9.7 ± 3.6 (15)	7.8 ± 4.2 (15)
Duration (min)	44.9 ± 12.9 (16)	51.2 ± 9.1 (15)	52.3 ± 9.6 (15)	42.9 ± 14.4 (15)	48.4 ± 10.8 (15)
Air exposure (min)					
Hopper	4.8 ± 1.6	7.2 ± 2.5	5.9 ± 0.9	3.5 ± 0.9	4.4 ± 2.2
Begin of sorting	8.3 ± 2.3	15.2 ± 3.2	7.0 ± 3.0	7.7 ± 3.0	6.0 ± 1.8
Mid	16.5 ± 3.3	19.6 ± 0.2	10.0 ± 2.7	9.2 ± 2.7	7.7 ± 1.3
End	n/a	n/a	12.7 ± 2.4	10.7 ± 2.4	9.7 ± 1.3
Sorting time (min)	23.2 ± 6.2 (6)	25.9 ± 6.8 (6)	13.0 ± 2.4 (3)	18.0 ± 10.3 (3)	11.7 ± 0.5 (3)
Environmental					
Wind force (Bft)	2.1 ± 0.7 (16)	4.8 ± 1.4 (15)	2.6 ± 0.8 (15)	2.7 ± 0.8 (15)	3.9 ± 0.8 (15)
Wave height (cm)	62.4 ± 9.9 (9)	106.8 ± 45.9 (5)	50.6 ± 14.2 (12)	36.4 ± 6.01 (4)	117.4 ± 18.9 (3)
Air temperature (°C)	10.9 ± 0.1 (6)	8 ± 0.4 (6)	8.1 ± 0.4 (3)	12.8 ± 0.2 (4)	17.0 ± 0.2 (3)
Seawater temperature (°C)	11.7 ± 0.1 (6)	7 ± 0.1 (6)	5.3 ± 0.1 (3)	14.4 ± 0.2 (4)	16.9 ± 0.1 (3)
Biological					
Total catch (kg)	2012.3 ± 1854.8 (6)	n/a	481.3 ± 440.9 (3)	333.5 ± 233.7 (4)	349.1 ± 105.1 (3)
TL of plaice (cm)	21.5 ± 3.7 (79)	19.5 ± 4.2 (57)	23.6 ± 3.2 (59)	20.6 ± 2.8 (60)	23.0 ± 3.1 (60)
TL of plaice (cm)—controls	n/a	22.6 ± 4.0 (20)	23.2 ± 2.9 (20)	n/a (20)	n/a

n/a, not available.

catch volumes in pulse trawls may have resulted in reduced codend compression underwater, in shorter sorting times and reduced air exposures on deck, all of which may have had a positive influence on sole vitality (Depestele *et al.*, 2014a). However, deviations from the sampling protocol may have confounded results in Experiment 2. Sole that were collected on-board the pulse trawler may have recovered from any impairment by being kept longer in a water-filled bucket than sole sampled on-board the conventional trawler before reflex testing. Holding organisms in water can contribute to their recovery (Yochum *et al.*, 2015). For example, impairment of sockeye salmon after 2-min air exposure was significantly reduced by holding fish for 15 min inside a flow-through container (Nguyen *et al.*, 2014).

Apart from this difference in on-board handling, the two different observers may have inconsistently scored reflexes. Despite having received laboratory-based training in what makes a reflex clear and strong ("present") as opposed to a weak, doubtful, or "absent" reflex response, observers may have interpreted protocol criteria differently in the field. Nevertheless, both scorers consistently recorded an increase in reflex impairment of plaice with increased air exposure periods. Despite a flatfish's capacity to breathe via their skin (Steffensen *et al.*, 1981), yellowtail flounder, another Pleuronectid species, was susceptible to death after trawl capture if exposed to air for 15 or 30 min (Barkley and Cadrin, 2012).

Our findings highlight the cumulative effects of capture-and-handling stress (Davis, 2002). For example, while unstressed plaice may be capable of withstanding 15–30 min air exposure, combination of air exposure with being exhausted from trawl capture may have cumulative detrimental effects. Abrupt

Table 3. List of candidate GLMM fitted to the survival data of discarded plaice (*P. platessa*) monitored in captivity for at least 14 d.

Model (variables included)	Δ_i *AIC*	ω_i
M1		
VS, TL, trawl duration, wave height, Δ temperature, Interaction: trawl duration : wave height	0	0.52
M2		
VS, TL, trawl duration, air exposure, wave height, Δ temperature Interactions: trawl duration : air exposure, trawl duration : wave height	2.37	0.16
M3		
VS, TL, trawl duration, air exposure, wave height, Δ temperature Interactions: trawl duration : air exposure, trawl duration : wave height, Δ temperature : air exposure	2.84	0.12
M4		
VS, TL, trawl duration, air exposure, wave height, Δ temperature Interactions: trawl duration : wave height, air exposure : wave height, Δ temperature : air exposure	4.35	0.06
M5		
VS, TL, trawl duration, air exposure, wave height, Δ temperature	4.46	0.06

Models (M1–M5) are shown with Akaike weights $\omega_i > 0.05$, calculated based on Δ_i *AIC* values. Models with higher Akaike weights ω_i have more empirical support. All models included the given variables' main effects (vitality score—VS and total length—TL, difference between surface seawater and air temperature—Δ, among others), and random effects for trawl and container. Interactions: trawl duration: air exposure, trawl duration: wave height, air exposure: wave height, and Δ temperature: air exposure.

Table 4. Significance of variables (fixed effects) and their interactions of generalized linear mixed models (GLMM, models M1–M5) with most empirical support (Akaike weights $\omega_i > 0.05$).

	M1	M2	M3	M4	M5
Vitality score	***	***	***	***	***
TL	***	***	***	***	***
Trawl duration (DU)	**	**	**	**	**
Wave height (WH)	○	○	○	○	○
Temperature difference (TD)	**	**	○	○	○
Air exposure (AE)	–	○	○	○	○
DU × WH	**	**	**	**	**
DU × AE	–	○	○	–	○
TD × WH	–	*	–	○	–
TD × AE	–	–	○	○	○
AE × WH	–	–	–	○	○

GLMMs were fitted to the survival data of discarded plaice (*P. platessa*) which were monitored in captivity for at least 14 d.
○ $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

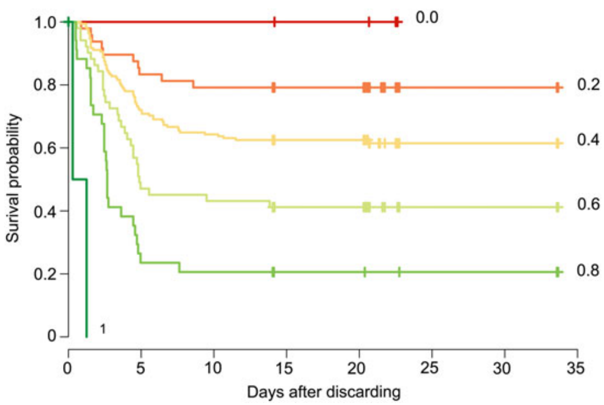

Figure 6. Non-parametric Kaplan–Meier survival probability estimates over days of monitoring of discarded plaice (*P. platessa*) at 0.2 vitality score intervals. The vitality score of injured and fully impaired individuals equals 1.

temperature changes by being hauled out of water into air seemed another relevant factor. Van Beek *et al.* (1990) observed higher survival at cooler days in winter when temperature differences between air and water are small. Such cumulative effects exemplify why interactions between, for example, trawl duration and wave height have greater explanatory power than just air exposure by itself.

Our results illustrate that a vitality score and TL were the most relevant explanatory variables to predict post-release survival probability of plaice. In agreement with other post-release survival studies (Yochum *et al.*, 2015), 14 d of post-release monitoring was appropriate to capture almost all fishing-related mortality events. Although one fish died after 21 d, >60% of mortalities occurred within the first 4 d. Reflexes of both plaice and sole were sensitive to capture stress, in particular air exposure, although some of the differences may have been related to an observer effect. As with other animal behaviour scores, reducing a continuous spectrum of responses to presence/absence observations to improve practicality (Cooke *et al.*, 2013) require a well-defined protocol, and assessments of bias, especially when multiple observers are involved (i.e. inter-observer reliability, Tuyttens *et al.*, 2014). Although scoring binary as opposed to ordinal or continuous responses removes some subjectivity in interpretation (Tuyttens *et al.*, 2009), it may still persist by abstracting from a continuous scale (Tuyttens *et al.*, 2014). Further research is needed to disentangle the effects of observer, and expectation bias on reflex impairment scores, especially in studies where more than one scorer is involved. Accuracy of scores may also be improved, if researcher handling periods before reflex (and injury) assessments are kept consistently as short as possible. Finally, the utility of RAMP as a proxy to predict post-release survival will depend on both laboratory-based and field calibration studies where key technical, environmental, and biological drivers of post-release survival are included.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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